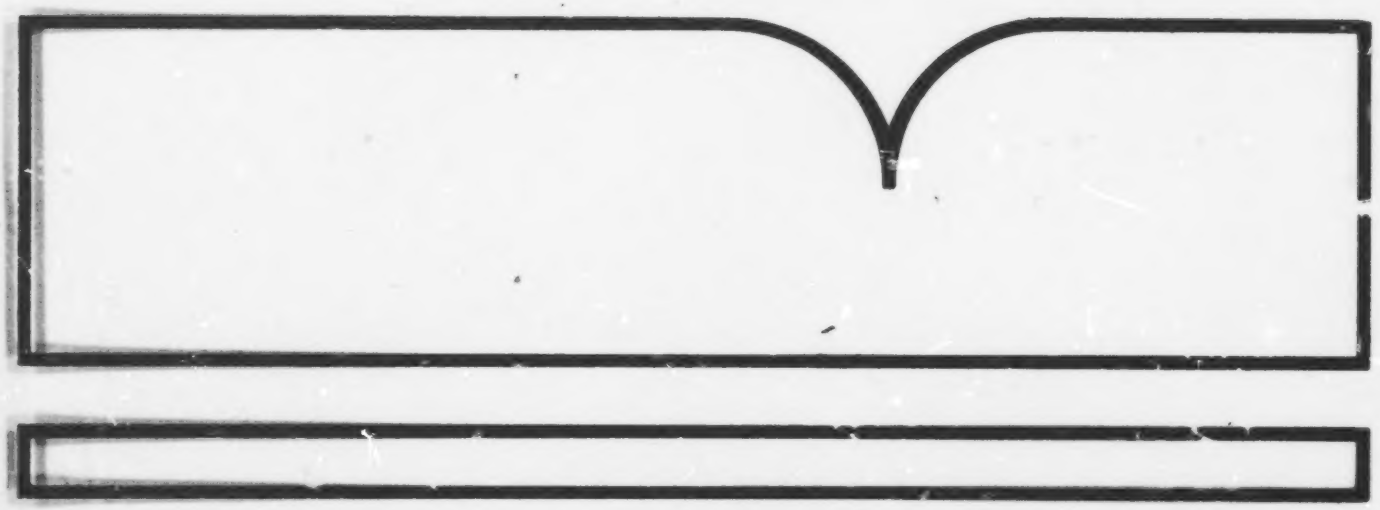
PB90-146176

Effect of Lindane on Hormonal Control of Reproductive Function in the Female Rat

(U.S.) Health Effects Research Lab., Research Triangle Park, NC

1989



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before compl

1. REPORT NO.	2.	PB90-146176
EPA/600/J-89/213		

4. TITLE AND SUBTITLE
Effect of Lindane on Hormonal Control of Reproductive Function in the Female Rat.

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
R.L.Cooper[1], R.W. Chadwick[1], G.L. Rehnberg[1], J.M. Goldman[2], K.C.Booth[2], J.F.Hein[1], W.K.McElroy[1]

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
1 EPA, HERL, RTP, NC
2 NSI, RTP, NC

10. PROGRAM ELEMENT NO.
ANNAIE

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory-RTP, NC
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

13. TYPE OF REPORT AND PERIOD COVERED
Journal Article

14. SPONSORING AGENCY CODE
EPA/600/11

15. SUPPLEMENTARY NOTES
Toxicology & Applied Pharmacology 99:384-394, 1989

16. ABSTRACT

The effect of the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH), lindane, on reproductive funtion in the female rat was examined in two experiments. In the first experiment, chronic treatment with 0, 5, 10, 20, and 40 mg/kg lindane delayed vaginal opening and disrupted ovarian cyclicity until approximately 110 days of age. Thereafter regular ovarian cycles were present in the majority of females in all dose groups. When sacrificed on the day of vaginal proestrus, the females receiving the two higher doses of lindane had smaller pituitary and uterine weights, lower serum and pituitary luteinizing hormone (LH) and prolactin and higher pituitary follicle stimulating hormone (FSH) concentrations than the oil-treated control females. Serum estrogen concentrations were not different from controls in the 5 and 20 mg/kg groups, significantly greater than the controls in the 10 mg/kg group and significantly less than the controls in the group receiving 40 mg/kg.

In a second experiment, the uterine weight and pituitary hormone response to a 10 µg injection of estradiol benzoate (EB) to 28-day-old, lindane-treated females was investigated. The uterine weights of the lindane treated prepubertal females were significantly lower than controls at 30 hs after EB injection. Furthermore, the EB-induced increase in serum luteinizing hormone, observed at 30 hs after EB injection, was lower in the lindane treated animals. Similarly, the reduction in pituitary LH, FSH and prolactin induced by EB was not as great in the lindane-treated animals as in the controls. Serum estrogen concentrations in the lindane-treated animals were not different from controls. These data indicate that lindane may effectively block the response of estrogen-dependent tissues to this ovarian steroid hormone and that this apparent antiestrogenic effect of lindane is responsible for the disturbances observed in the neuroendocrine control of ovarian function in the rat.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	12
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77)

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

TOXICOLOGY AND APPLIED PHARMACOLOGY 99, 384–394 (1989)

EPA/600/J-89/213

Effect of Lindane on Hormonal Control of Reproductive Function in the Female Rat[1]

RALPH L. COOPER,* ROBERT W. CHADWICK,† GEORGIA L. REHNBERG,* JEROME M. GOLDMAN,‡ KIMBERLY C. BOOTH,‡ JOY F. HEIN,* AND W. KEITH MCELROY*

**Endocrinology and Gerontology Section and †Genetic Toxicology Section, Health Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, North Carolina 27711; and ‡NSI Technology Services, Environmental Sciences, Research Triangle Park, North Carolina 27709*

Received July 18, 1988; accepted March 19, 1989

Effect of Lindane on Hormonal Control of Reproductive Function in the Female Rat. COOPER, R. L., CHADWICK, R. W., REHNBERG, G. L., GOLDMAN, J. M., BOOTH, K. C., HEIN, J. F., AND MCELROY, W. K. (1989). *Toxicol. Appl. Pharmacol.* **99**, 384–394. The effect of the γ isomer of 1,2,3,4,5,6-hexachlorocyclohexane, lindane, on reproductive function in the female rat was examined in two experiments. In the first experiment, chronic treatment with 0, 5, 10, 20, and 40 mg/kg lindane delayed vaginal opening and disrupted ovarian cyclicity until approximately 110 days of age. Thereafter, regular ovarian cycles were present in the majority of females in all dose groups. When killed on the day of vaginal proestrus, the females receiving the two higher doses of lindane had smaller pituitary and uterine weights, lower serum and pituitary luteinizing hormone (LH) and prolactin, and higher pituitary follicle stimulating hormone (FSH) concentrations than the oil-treated control females. Serum estrogen concentrations were not different from controls in the 5 and 20 mg/kg groups, significantly greater than the controls in the 10 mg/kg group, and significantly less than the controls in the group receiving 40 mg/kg. In a second experiment, the uterine weight and pituitary hormone response of 28-day-old, lindane-treated females to a 10-μg injection of estradiol benzoate (EB) were investigated. The uteri of the lindane-treated prepubertal females were smaller than controls at 30 hr after EB injection. Furthermore, the EB-induced increase in serum luteinizing hormone, observed at 30 hr after EB injection, was lower in the lindane-treated animals. Similarly, the reduction in pituitary LH, FSH, and prolactin induced by EB was not as great in the lindane-treated animals as in the controls. Serum estrogen concentrations in the lindane-treated animals were not different from controls. These data indicate that lindane may effectively block the response of estrogen-dependent tissues to this ovarian steroid hormone and that this apparent antiestrogenic effect of lindane is responsible for the disturbances observed in the neuroendocrine control of ovarian function in the rat. © 1989 Academic Press, Inc.

The gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH), lindane, is present in a wide of variety agricultural, medical, and veterinary products (Wattal, 1983; Ullmann, 1972; Solomon *et al.*, 1977). The adverse health effects of lindane have been studied extensively. The principal responses of exposure to lindane in animals appear to be neurological and behavioral (Hulth *et al.*, 1976; Joy, 1982), although alterations in hepatic and kidney function have also been documented (Dikshith *et al.*, 1978). Lindane has been shown to disrupt reproductive function

[1] The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

0041-008X/89 $3.00
Copyright © 1989 by Academic Press, Inc.
All rights of reproduction in any form reserved.

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

in birds (Chakravarty and Lahari, 1986) and in mammals. In the rat, testicular atrophy and degenerative changes in the seminiferous tubules have been reported after exposure to lindane (Datta and Dikshith, 1973; Dikshith *et al.*, 1978; Nigam *et al.*, 1979; Shivanandappa and Krishnakumari, 1983; Chowdhury *et al.*, 1987). In the female rat, lindane has also been reported to alter reproductive development (Naishtein and Leibovich, 1971) and the reproductive process in the adult (Lahire *et al.*, 1985; Uphouse, 1987).

The exact mechanism(s) of lindane-induced reproductive changes is not well understood, yet there are several effects of this compound that make it reasonable to assume that it would alter reproductive function. Lindane-induced convulsions are believed to be associated with this compound's ability to block neurotransmission at the γ-aminobutyric acid (GABA) receptor–chloride channel complex (Joy and Albertson, 1987), and/or with changes in central nervous system calcium regulation of transmitter release (Woolley *et al.*, 1985; Joy, 1982; Aldegunde *et al.*, 1986). It is, therefore, possible that the neuroendocrine control of gonadal function is impaired due to alterations in the limbic system and/or hypothalamic control of pituitary hormone secretion. Alternatively, lindane could affect reproductive function in the female by altering hepatic steroid metabolism and reducing serum estrogen (Peakall, 1970; Welch *et al.*, 1971). Thus, it is possible that the feedback regulation of pituitary gonadotropin secretion by the ovarian steroid hormones is altered as a consequence of shifts in serum estrogen and/or progesterone levels. However, direct measurements of pituitary and ovarian steroid hormones in the female rat exposed to lindane have not been reported.

Since a detailed analysis of the hormonal control of reproductive function in the female rat exposed to lindane is lacking, the purpose of this study was to evaluate the changes in pituitary and gonadal hormones following treatment with different doses of lindane and provide further insight into the mechanisms associated with this insecticide's adverse effects on reproductive function.

METHODS

Experiment 1. Female pups obtained from timed-pregnant Fischer-344 rats were assigned randomly to the various treatment groups at 21 days of age and maintained at 22 ± 2°C on a 12:12 hr light:dark schedule (lights on at 0700 hr). Beginning on Day 21, each rat was gavaged daily with 0, 5, 10, 20, or 40 mg/kg lindane in peanut oil. Lindane was obtained from the Analytical Reference Standards Repository of the USEPA Health Effects Laboratory (Research Triangle Park, NC). The dosing volume was continuously adjusted to 0.1% of the animal's body weight. Body weights and food consumption (Purina Lab Chow 5001) were recorded daily. Beginning on Day 28, each female was examined for vaginal opening. Thereafter, daily vaginal smears were obtained and classified as either diestrus (primarily leukocytes), proestrus (nucleated epithelial cells), or estrus (cornified epithelial cells).

Animals showing at least two consecutive regular ovarian cycles between 110 and 125 days of age were decapitated at 1300 hr on the day of vaginal proestrus. Trunk blood was collected and stored in glass vials until analyzed for luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), estradiol, and progesterone according to the procedures described below. The pituitary was removed, weighed, and sonicated in 1 ml Medium 199/0.2% bovine serum albumin (Medium 199/BSA, pH adjusted to 7.4) and stored at −55°C until assayed for LH, PRL, and FSH. The uterus and ovaries were removed, trimmed of fat and weighed.

Experiment 2. In order to evaluate better the effects of lindane on the pituitary and uterine responses to estrogen, we investigated the effect of this pesticide on the reproductive system of the immature female rat using the procedure originally described by Astwood (1938), which allows the assessment of short-term lindane effects on the uterine response to estrogen and provides information on the threshold levels and graded estrogenic activity of a compound. This bioassay thus provided a means to evaluate the suspected interference of lindane with the action of exogenous estradiol.

Sixty-four female pups were assigned to one of eight treatments. Four of these groups were gavaged with peanut oil and four groups were gavaged with lindane in peanut oil at a dose of 30 mg/kg. This dose was selected on the basis of the results from Experiment 1. Treatment was initiated at 21 days of age and continued daily until the end of the experiment. At 27 days, two groups of lindane-treated and two groups of corn oil-treated animals were injected subcutaneously with estradiol benzoate

TABLE 1

SUMMARY OF GROUPINGS OF PREPUBERTAL FEMALES SHOWING TREATMENT AND TIME OF TERMINATION

Group[a]	N	Gavage beginning Day 21	0900 hr Day 27 subcutaneous	Time of decapitation (hr)[b]
Con/CO-6	8	Peanut oil	Corn oil	6
Con/CO-30	8	Peanut oil	Corn oil	30
Con/EB-6	8	Peanut oil	Estrogen	6
Con/EB-30	8	Peanut oil	Estrogen	30
L/CO-6	8	Lindane	Corn oil	6
L/CO-30	8	Lindane	Corn oil	30
L/EB-6	8	Lindane	Estrogen	6
L/EB-30	8	Lindane	Estrogen	30

[a] Con, control treated with peanut oil; CO, corn oil; L, lindane.
[b] Expressed as hours since time of EB injection.

(EB, 10 μg/pup in 0.1 ml corn oil), while the remaining animals were injected with the corn oil only. Groups of animals ($n = 8$) from each of the lindane/peanut oil and estradiol/corn oil conditions were then decapitated at 6 or 30 hr after EB or corn oil treatment. The trunk blood and pituitaries were then processed for hormonal analysis as described above. The uteri and ovaries were removed, trimmed of fat, and weighed. An outline of this experimental design is presented in Table 1.

Hormone assays. LH, FSH, and PRL were all analyzed by radioimmunoassay using materials supplied by the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases as detailed elsewhere (Cooper *et al.*, 1987). The assay sensitivities of LH and FSH were increased by a 24-hr co-incubation of sample and first antibody prior to the addition of the labeled hormone. The respective sensitivities, inter- and intraassay variances were 15 pg/tube, 7.8% and 5.5% for LH; 150 pg/tube, 7.7% and 6.9% for FSH, and 32 pg/tube, 6.5% and 2.6% for PRL.

Serum estradiol and progesterone were determined using radioimmunoassay kits obtained from Diagnostic Products Corp. (Los Angeles).

Statistics. Significant effects among groups were tested by analysis of variance under the General Linear Models procedure of the Statistical Analysis System (SAS) package, with the α level set at 0.05. Significant F values were then compared further among groups by Student's t test. Differences in the patterns of vaginal cycling among the groups were compared using the Fisher Exact test.

RESULTS

Experiment 1

Effect of lindane on vaginal opening and vaginal cycles. The mean age of vaginal opening in females treated with 5 and 20 mg/kg of lindane was not different from that of the control females, while animals receiving 10 and 40 mg/kg lindane showed significant delays in vaginal opening (Table 2). The delay

TABLE 2

AGE AND BODY WEIGHT ON THE DAY OF VAGINAL OPENING

	Treatment				
	Control	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
N	6	6	8	11	12
Age (days)	36.50 ± 1.52	38.83 ± 1.18	40.50 ± 1.12[a]	39.50 ± 1.06	43.50 ± 1.89[a]
Body wt (g)	86.63 ± 3.23	91.92 ± 3.54	95.81 ± 3.04	94.09 ± 1.91[a]	99.29 ± 3.84[a]

[a] $p < 0.05$ for comparisons between treated and control. Data are expressed as means ± SEM.

TABLE 3

PROPORTION OF ANIMALS REVEALING REGULAR 5-DAY ESTROUS CYCLES DURING TREATMENT WITH LINDANE

Dose (mg/kg)	Days			
	51–70	71–90	91–110	111–130
0	6/6	5/6	6/6	6/6
5	2/6*	2/6*	5/6	5/6
10	1/8*	1/8*	3/8*	7/8
20	2/10*	1/10*	4/10*	9/9
40	0/11*	0/9*	0/6*	4/6

* $p < 0.02$ for comparisons between treated and control groups.

in vaginal opening was not the consequence of lowered body weight in the treated animals, as the weights of the females treated with 20 and 40 mg/kg lindane were actually greater than those of the controls on the day of vaginal opening.

After vaginal opening, lindane delayed the appearance of regular vaginal cycles (Table 3). However, the majority of females in the 5 mg/kg group did show regular 5-day cycles after 90 days of age, and most in the three higher dose groups showed regular 5-day cycles after 110 days of age. The vaginal smear pattern present before regular cycling appeared was mixed. That is, in all dose groups there were females that had extended periods during which the daily smears were leukocytic (i.e., 5–7 days, persistent diestrus), while other females had smears that remained cornified for 3–4 consecutive days (e.g., persistent estrus). There was no apparent dose–response relationship between the vaginal cytology observed and the dose of lindane administered, and several animals in each dose group showed periods of both persistent estrus and persistent diestrus.

The body and organ weights of the females on the day of decapitation are shown in Table 4. The mean body weights of the 20 and 40 mg/kg dose groups were significantly greater than those of the controls. The mean uterine weights of the 20 and 40 mg/kg groups were significantly lower than those in control females. This was true whether the uterine weights were expressed as absolute tissue weights or as percentages of body weight. However, when expressed as a percentage of body weight, the mean uterine value for the 5 mg/kg group was also significantly smaller than that of the controls, while that of the 10 mg/kg dose group was not different. The mean pituitary weights for the 10 and 40 mg/kg groups were also significantly less than that for controls. When pituitary weights were expressed as a percentage of the female's body weight, the mean values of the 10, 20, and 40 mg/kg groups were significantly lower than those of the controls.

Reproductive hormone concentrations during proestrus. Serum LH and prolactin concentrations at the time of decapitation were significantly lower in females receiving the two higher doses of lindane, while serum FSH was similar to controls in all dose groups (Fig. 1). Pituitary LH in the 20 and 40 mg/kg groups was less than that of controls, while pituitary FSH was significantly greater. The concentration of prolactin in the pituitaries of the 40 mg/kg group was significantly lower than controls.

Serum progesterone levels were close to basal levels for the estrous cycle and were not different among the groups observed (Fig. 2). The mean serum estradiol concentration in females receiving the 10 mg/kg dose of lindane was significantly greater than controls. The mean estradiol concentrations in the 5 and 20 mg/kg groups were not different from controls, but were significantly lower in the 40 mg/kg group.

Experiment 2

Effect of lindane on the prepubertal female's response to estrogen. Treatment of the 27-day-old prepubertal females with EB increased the uterine weights at 6 and 30 hr after injection in both the control and lindane-

TABLE 4

ORGAN WEIGHTS (MEANS ± SEM) FOR ANIMALS IN EXPERIMENT 1 ON THE DAY OF TERMINATION

		Pituitary		Uterus		Ovaries	
Dose	Body weight	Weight (mg)	Percentage body wt	Weight (mg)	Percentage body wt	Weight (mg)	Percentage body wt
0	186 ± 7	11.6 ± 0.6	0.063 ± 0.002	614.9 ± 25.2	0.329 ± 0.008	44.6 ± 4.5	0.024 ± 0.002
5	190 ± 3	10.4 ± 0.5	0.055 ± 0.003	574.4 ± 19.7	0.304 ± 0.008*	40.7 ± 3.5	0.022 ± 0.002
10	187 ± 4	10.0 ± 0.3*	0.054 ± 0.002*	563.6 ± 41.0	0.303 ± 0.022	39.3 ± 1.2	0.021 ± 0.001
20	230 ± 6*	11.4 ± 0.5	0.050 ± 0.001*	448.1 ± 33.5*	0.197 ± 0.015*	39.6 ± 1.2	0.017 ± 0.001*
40	219 ± 2*	9.4 ± 0.6*	0.045 ± 0.002*	220.7 ± 23.1*	0.104 ± 0.007*	35.2 ± 2.0	0.017 ± 0.002*

* $p < 0.05$ for comparisons beween treated and control groups.

gavaged females (Table 5). Also, at 6 hr after EB treatment, the uterine weights of the control and lindane-gavaged females were not different. However, the mean uterine weight of the control animals killed at 30 hr after EB was significantly greater than that observed in the lindane-gavaged animals. Body weights were comparable among the treated and control groups.

At 6 hr, the pituitaries of the females receiving lindane alone weighed less than those of the females receiving peanut oil alone, but by 30 hr no such differences were seen. In the lindane- and peanut oil-gavaged animals, EB caused a significant increase in pituitary weights at 30 hr. But in this instance. the pituitary weights of the peanut oil females were greater than the females receiving lindane.

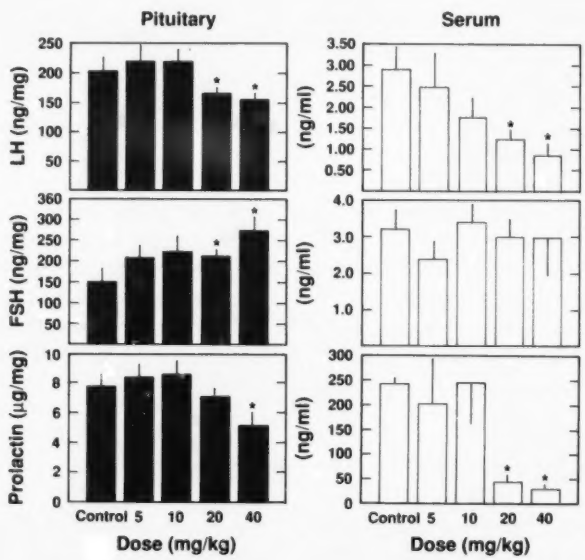


FIG. 1. Pituitary (solid bars) and serum (open bars) hormone concentrations (means ± SEM) of females treated with peanut oil (control) or lindane. All animals were killed at 1300 hr on the day of vaginal proestrus (*$p < 0.05$).

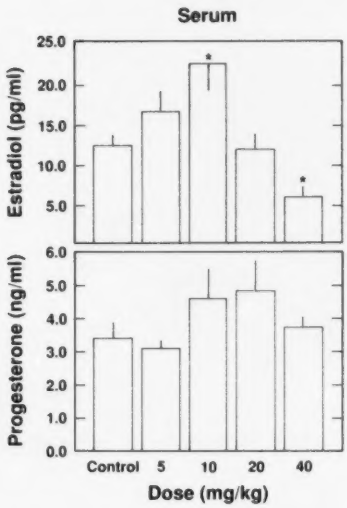


FIG. 2. Serum concentration (means ± SEM) of ovarian hormones in females studied in Experiment 1 (*$p < 0.05$).

No differences in serum estradiol were found among the groups at either 6 or 30 hr after EB injection (Table 5).

Effect of lindane on reproductive hormones in the prepubertal female. EB treatment caused significant changes in the concentrations of the serum and pituitary hormones. Compared to animals not receiving EB and decapitated at 30 hr, the concentrations of LH and FSH in the pituitaries of the control + estrogen- and lindane + estrogen-treated females were significantly lower (Fig. 3). However, at this time the concentrations of pituitary LH and FSH for the lindane-gavaged + EB animals were greater than those of the control + EB females. Compared to females not receiving EB, the pituitary prolactin concentrations were reduced at both 6 and 30 hr after EB in both control and lindane-gavaged females. However, as with LH and FSH, the pituitary prolactin in the lindane + EB group, killed at 30 hr, was significantly greater than that in the control + EB group killed at the same time.

There were no differences in the serum LH and prolactin concentrations of females receiving peanut oil or lindane and no EB treatment (Fig. 4). EB treatment did result in a significant increase of serum LH in both control and lindane-treated animals killed at 30 hr. However, at this time the serum LH of lindane-treated animals was less than that of controls. EB treatment also resulted in a significant increase in serum prolactin in the control group at 6 hr and in both control and

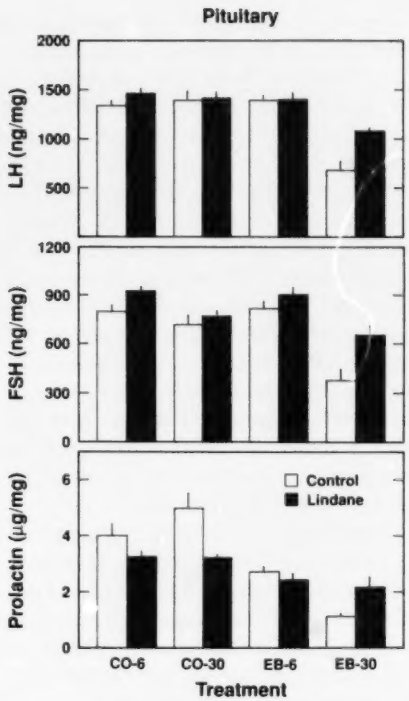
TABLE 5

BODY WEIGHTS, ORGAN WEIGHTS, AND SERUM ESTRADIOL VALUES (MEANS ± SEM) OBSERVED IN THE ANIMALS KILLED IN EXPERIMENT 2

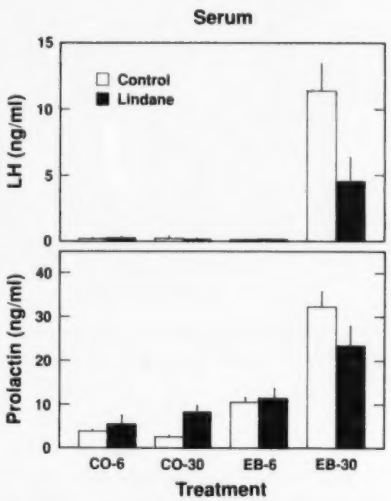
Group	Body weight	Pituitary weight	Uterine weight	Serum estradiol
Con/CO-6	65.57 ± 1.97	3.94 ± 0.06	64.7 ± 4.5	nd
Con/CO-30	69.43 ± 2.02	4.23 ± 0.24	63.9 ± 4.1	nd
Con/EB-6	61.00 ± 1.43	3.87 ± 0.12	85.1 ± 3.0[a]	190.3 ± 39.98
Con/EB-30	71.57 ± 2.23	4.77 ± 0.17[a]	139.1 ± 3.4[a]	109.5 ± 40.45
L/CO-6	61.00 ± 1.96	3.43 ± 0.15[b]	52.7 ± 1.8	nd
L/CO-30	65.00 ± 2.48	3.63 ± 0.11	58.3 ± 3.4	nd
L/EB-6	61.17 ± 2.61	3.62 ± 0.12	84.4 ± 7.4[a]	246.7 ± 36.3
L/EB-30	62.86 ± 2.19	4.16 ± 0.11[a,b]	108.1 ± 2.9[a,b]	49.6 ± 14.0

[a] Within lindane or peanut oil treatment conditions, the mean is significantly different ($p < 0.05$) from animals not receiving EB and killed at the same time.

[b] The mean of the lindane-treated animals is significantly different ($p < 0.05$) from the peanut oil-treated animals killed at the same time point and under the same EB treatment.



3



4

FIG. 3. Concentrations (means ± SEM) of pituitary LH, FSH, and prolactin of prepubertal females studied in Experiment 2. Animals were treated with peanut oil (control, open bars) or lindane (black bars) for 7 days. On Day 8, the females received estrogen (EB) or corn oil (CO) at 0900 hr and were decapitated 6 or 30 hr later. Comparisons:

LH	FSH	Prolactin
Con/EB-30 < Con/CO-30**	Con/EB-30 < Con/CO-30**	Con/EB-6 < Con/CO-6*
L/EB-30 < Con/CO-30**	L/EB-30 < L/CO-30*	Con/EB-30 < Con/CO-30**
Con/EB-30 < L/EB-30**	Con/EB-30 < L/EB-30*	L/EB-6 < L/CO-6*
		L/EB-30 < L/CO-30*
		L/CO-30 < Con/CO-30**
		Con/EB-30 < L/EB-30*

*$p < 0.05$
**$p < 0.01$

FIG. 4. Concentrations (means ± SEM) of serum LH and prolactin in prepubertal females studied in Experiment 2. See text and Table 1 for further explanation. Comparisons:

LH	Prolactin
Con/CO-30 < Con/EB-30**	Con/CO-6 < Con/EB-6**
L/CO-30 < L/EB-30**	Con/CO-30 < Con/EB-30**
L/EB-30 < Con/EB-30*	L/CO-30 < L/EB-30**

*$p < 0.05$
**$p < 0.01$

lindane-treated rats at 30 hr. Moreover, at this time, serum prolactin concentration of the control group was significantly greater than that of the lindane group.

DISCUSSION

The present study demonstrates that lindane can cause several alterations in the hormonal control of ovarian function. The delay in puberty and disruption of regular ovarian cycling have been reported previously (Naishtein and Leibovich, 1971; Lahire *et al.*, 1985). In the present study, we found that although irregular cycles were present in the majority of females prior to 90 days of age, a substantial number of females did show normal length (i.e., 4 or 5 day) vaginal cycles after approximately 110 days of age. The finding that the body weights of the females treated with 20 and 40 mg/kg/day were greater than those of the control animals may be related to the alterations in estrous cyclicity. The periodic increases of serum estrogen (which lead to a reduction of food intake and body weight) occurred less often in the treated animals displaying irregular cycles (Chadwick *et al.*, 1988). However, it may also be that lindane exerts an antagonistic effect on any endogenous estrogen that may be present in the blood. This possibility is suggested by a number of findings in the present study. First, estrogens have a well-documented uterotropic action, and although both the 20 and 40 mg/kg doses of lindane reduced uterine weight, only the 40 mg/kg dose significantly reduced serum estrogen levels. A possible lindane interference with the uterine response to estrogen is further supported by the observation that the uteri of the lindane + EB-treated prepubertal females were also smaller than control females. This effect of lindane has been reported previously (Welch *et al.*, 1971; Raizada *et al.*, 1980). These investigators concluded that the smaller uterine weights were attributable to a more rapid clearance of estrogen induced by lindane. However, serum estradiol was not measured. In the present study, 20 mg/kg of lindane did not alter endogenous estrogen levels in the intact cycling female, nor did this insecticide alter the levels of estradiol measured in the prepubertal female receiving 30 mg/kg and injected with EB, although in both cases the uterine weights of the lindane-gavaged females were less than controls. Finally, the serum estradiol concentration of the females receiving 10 mg/kg lindane in Experiment 1 was significantly higher than controls. We know of no previous reports suggesting that serum estradiol is elevated in response to lindane treatment. Whether this increase in response to 10 mg/kg is the consequence of altered metabolism or secretion of this hormone remains to be determined by a more thorough evaluation of the histological and endocrine changes associated with exposure to a range of lindane doses. However, in combination, these observations suggest that in addition to the numerous other effects reported for lindane, this compound may also possess an anti-estrogenic action. While this directly contrasts with the previously proposed estrogenicity of lindane, it should be noted that it is not unusual for a substance to have both estrogenic and anti-estrogenic properties (Wade and Blaustein, 1978). Recently, Uphouse (1987) found that lindane treatment on the morning of vaginal proestrus reduced sexual receptivity in the female rat. This effect did not appear to be the result of lindane's effect on central nervous system GABA transmission, as similar treatment with picrotoxin (a GABA-receptor blocking agent) had no effect. Anti-estrogens, such as monohydroxytamoxifen (a high-affinity anti-estrogen), have been shown to reduce sexual receptivity in the female rat (Meisel *et al.*, 1987). Such results would again argue that lindane possesses anti-estrogenic properties and that the time course for the suspected blockade of lindane may be more rapid than that implied by the present study.

The lindane-induced alterations in the concentrations of LH and prolactin in the se-

rum and pituitary and the effect of this substance on pituitary weight are also consistent with an anti-estrogenic effect. Estrogens (Wiklund *et al.,* 1981) and weakly estrogenic pesticides (Cooper *et al.,* 1986a) will cause an increase in pituitary weight. The pituitary weights (relative to the body weight) of the females in Experiment 1 receiving 10, 20, and 40 mg/kg lindane were less than those of the control females.

In the regularly cycling female, ovarian steroids have both a positive and a negative feedback action on the brain and pituitary to synchronize the ovulatory surge of LH as well as the proestrous rise in serum FSH and prolactin. Thus, the increase in circulating estradiol which occurs before the release of gonadotropins serves to increase the amount of the hormones in the pituitary which are available for release and enhances the pituitary's response to the CNS signals controlling their release. Both pituitary and serum LH and prolactin concentrations were decreased on the afternoon of vaginal proestrus in females receiving 20 and 40 mg/kg dose groups. This suggests that lindane interferes with the effect that estrogen would normally have on pituitary hormone synthesis and perhaps release. Serum FSH, on the other hand, was not different for the control and treated females in Experiment 1, while pituitary FSH concentrations were significantly elevated in the 20 and 40 mg/kg groups. This observation could argue against any suggested anti-estrogenic action of lindane. However, the effect of lindane on FSH requires further study. We have previously observed differences in the direction and amount of change in LH versus FSH concentration and release following treatment with reproductive toxins such as diethylstilbesterol (Cooper *et al.,* 1986b) and the benomyl metabolite, carbendazim (Goldman *et al.,* 1989). Such results imply that some toxins exert a differential effect on the control of LH and FSH. This differential effect on the gonadotropins may involve changes in the regulation of the gonadal feedback control of FSH secretion.

In the prepubertal female, a single injection of estrogen will induce a rise in serum LH and prolactin by the following afternoon. As LH and prolactin increase in the serum, the concentrations of these hormones in the pituitary decrease. In Experiment 2, lindane-gavaged females had lower serum LH concentrations and greater pituitary LH, FSH, and prolactin concentrations, suggesting that lindane prevented the estrogen-induced release of these hormones. Also, the data from Experiment 2 show that, with the possible exception of pituitary prolactin on Day 28, lindane treatment alone did not alter pituitary or serum hormone levels. Thus, it appears that estrogen was required to expose the difference between the control and treated animals.

The changes in pituitary hormone concentration and release observed in the prepubertal females (Experiment 2) are consistent with the finding that lindane will delay vaginal opening. Prolactin, LH and FSH are all important to the normal development of puberty (Ojeda *et al.,* 1980). It remains to be determined whether lindane acts directly on the hypothalamic and pituitary control of puberty, or on the synthesis and release of the pituitary hormones in response to estrogen feedback. Since the major difference between the lindane and control females in this study was observed only after EB injection, the results further suggest that lindane blocked the pituitary's response to this steroid hormone. However, it would be inappropriate to rule out at this time a direct CNS or pituitary action of lindane on pubertal development.

The fact that there are several changes in the hormonal control of ovarian function following lindane treatment suggests that exposure to this pesticide should affect fertility in the female rat. Palmer *et al.* (1978) reported no reproductive or teratotogenic effects in rats fed dietary lindane (25, 50, and 100 ppm). On the other hand, Petrescu *et al.* (1974) reported that lindane (5, 10, and 15 mg/kg diet) prolonged pregnancy and decreased fertility. The results of this latter study may reflect the effect of lindane on the

hormonal support of pregnancy and lactation, as the suggested anti-estrogenic action of this insecticide may interfere with the endocrine support of uterine and luteal function during pregnancy and/or the subsequent milk production during nursing.

In summary, our data suggest that lindane has anti-estrogenic properties, interfering with the effect of circulating estradiol on target tissues. A characterization of the particular histological changes and of the potential alterations in receptor function involved in this suspected anti-estrogenic effect of lindane remain to be determined.

ACKNOWLEDGMENTS

We thank Ms. Debbie Crawford and Bette Terrill for their excellent technical assistance in conducting this research.

REFERENCES

ALDEGUNDE, M., MIGUEZ, I., PARAFITA, M., AND DURAN, R. (1986). Effect of lindane on brain monoamine metabolism. *Gen. Pharmacol.* **17,** 633–635.

ASTWOOD, E. B. (1938). A six-hour assay for the quantitative determination of estrogen. *Endocrinology* **23,** 25–32.

CHADWICK, R. W., COOPER, R. L., CHANG, J., REHNBERG, G. L., AND MCELROY, W. K. (1988). Lindane-induced obesity and lesions in the reproductive system of female Fisher 344 rats. *The Toxicologist* **8,** 237.

CHAKRAVARTY, S., AND LAHIRI, P. (1986). Effect of lindane on eggshell characteristics and calcium level in the domestic duck. *Toxicology* **42,** 245–258.

CHOWDHURY, A. R., VENKATAKRISHNA-BHATT, H., AND GAUTAM, A. K. (1987). Testicular changes of rats under lindane treatment. *Bull. Environ. Contam. Toxicol.* **38,** 154–156.

COOPER, R. L., GOLDMAN, J. M., AND REHNBERG, G. L. (1986a). Pituitary following treatment with reproductive toxins. *Environ. Health Perspect.* **70,** 177–184.

COOPER, R. L., GOLDMAN, J. M., REHNBERG, G. L., HEIN, J. F., AND MCELROY, W. K. (1986b). Altered response to a reproductive toxin in the aging male rat. *The Toxicologist* **6,** 288.

COOPER, R. L., GOLDMAN, J. M., REHNBERG, G. L., MCELROY, W. K., AND HEIN, J. F. (1987). Effects of metal cations on pituitary hormone secretion *in vitro*. *J. Biochem. Toxicol.* **2,** 241–249.

DATTA, K. K., AND DIKSHITH, T. S. S. (1973). Histopathologic changes in the testis and liver of rats repeatedly exposed to pesticides. *Exp. Pathol.* **8,** 63–370.

DIKSHITH, T. S. S., TANDON, S. K., DATTA, K. K., GUPTA, P. K., AND BEHARI, J. R. (1978). Comparative response of male rats to parathion and lindane: Histopathological and biochemical studies. *Environ. Res.* **17,** 1–9.

GOLDMAN, J. M., REHNBERG, G. L., COOPER, R. L., GRAY, L. E., HEIN, J. F., AND MCELROY, W. K. (1989). Effects of the benomyl metabolite, Carbendazim, on the hypothalamic-pituitary reproductive axis in the male rat. *Toxicology,* in press.

HULTH, L., LARSSON, R., CARLSSON, R., AND KIHLSTROM, J. E. (1976). Convulsive action of small single oral doses of the insecticide lindane. *Bull. Environ. Contam. Toxicol.* **16,** 133–137.

JOY, R. M. (1982). Mode of action of lindane, dieldrin and related insecticides in the central nervous system. *Neurobehav. Toxicol. Teratol.* **4,** 813–823.

JOY, R. M., AND ALBERTSON, T. E. (1987). Interactions of lindane with synaptically mediated inhibition and facilitation in the dentate gyrus. *Neurotoxicology* **8,** 529–542.

LAHIRI, P., CHAKRAVARTY, S., MONDAL, A., AND SIRCAR, S. (1985). Effect of lindane on cytology and cytochemistry of exfoliated vaginal cells. *Exp. Clin. Endocrinol.* **87,** 303–308.

MEISEL, R. L., DOHANICH, G. P., MCEWEN, B. S., AND PFAFF, D. W. (1987). Antagonism of sexual behavior in female rats by ventromedial hypothalamic implants of antiestrogen. *Neuroendocrinology* **45,** 201–207.

NAISHTEIN, S. YA., AND LEIBOVICH, D. L. (1971). Effect of small doses of DDT and lindane and their mixture on sexual function and embryogenesis in rats. *Hyg. Sanit.* **36,** 190–195.

NIGAM, S. K., LAKKAD, B. C., KARNIK, A. B., THAKORE, K. N., BHATT, D. K., BABU, K. A., AND KASHYAP, S. K. (1979). Effect of hexachlorocyclohexane feeding on testicular tissue of pure inbred Swiss mice. *Bull. Environ. Contam. Toxicol.* **23,** 431–437.

OJEDA, S. R., ANDREWS, W. W., ADVIS, J. P., AND SMITH-WHITE, S. (1980). Recent advances in the endocrinology of puberty. *Endocrinol. Rev.* **1,** 228–257.

PALMER, A. K., COZENS, D. D., SPICER, E. J. F., AND WORDEN, A. N. (1978). Effects of lindane upon reproductive function in a 3-generation study in rats. *Toxicology* **10,** 45–54.

PEAKALL, D. B. (1970). p,p'-DDT: Effect on calcium metabolism and concentration of estradiol in the blood. *Science* **168,** 592–594.

PETRESCU, S. T., DOBRE, V., AND LEIBOVICI, M. (1974). Cercetari privind effectele administrarii prelungite a unor substante pesticide organochlorate (lindan, DDT), asupra sobolanului alb day laborator. *Rev. Med.-Chirurg. Soc. Med. Nat.* **78,** 831–842.

RAIZADA, R. B., MISRA, P., SAXENA, I., DATTA, K. K., AND DIKSHITH, T. S. S. (1980). Weak estrogenic activity of lindane in rats. *J. Toxicol. Environ. Health* **6,** 483–492.

SHIVANANDAPPA, T., AND KRISHNAKUMARI, M. K. (1983). Hexachlorocyclohexane-induced testicular dysfunction in rats. *Acta Pharmacol. Toxicol.* **52,** 12–17.

SOLOMON, L. W., FAHRNER, L., AND WEST, D. P. (1977). Gamma benzene hexachloride toxicity. *Arch. Dermatol.* **113,** 353–357.

ULLMAN, E. (1972). *Lindane. Monograph of an Insecticide.* Verlag K Scillinger, Freiburg im Breisgau, West Germany.

UPHOUSE, L. (1987). Decreased rodent sexual receptivity after lindane. *Toxicol. Lett.* **39,** 7–14.

WADE, G. N., AND BLAUSTEIN, J. D. (1978). Effects of an anti-estrogen on neural estradiol binding and on behaviors in female rats. *Endocrinology* **102,** 245–251.

WATTAL, B. L. (1983). The role and use of pesticides in health practices. *Pestic. Inform.* **9,** 43.

WELCH, R. M., LEVIN, W., KUNTZMAN R., JACOBSON, M., AND CONNEY, A. H. (1971). Effects of halogenated hydrocarbon insecticides on the metabolism and uterotropic action of estrogens in rats and mice. *Toxicol. Appl. Pharmacol.* **19,** 234–246.

WIKLUND, J., WERTZ, N., AND GORSKI, J. (1981). A comparison of estrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzman rats. *Endocrinology* **109,** 1700–1707.

WOOLLEY, D., ZIMMER, L., DODGE, D., AND SWANSON, K. (1985). Effects of lindane-type insecticides in mammals: Unsolved problems. *Neurotoxicology* **6,** 165–192.